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ORIGINAL ARTICLE

Identification of TAAR5 Agonist Activity of Alpha-NETA and Its Effect on Mismatch Negativity Amplitude in Awake Rats

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Abstract

Mismatch negativity (MMN) is a well-defined component of human event-related potentials that reflects the pre-attentive, stimulus-discrimination process and is associated with involuntary switching of attention. MMN-like responses detected in animal models provide an opportunity to investigate the neural mechanisms of this process that involves several neurotransmitter and neuromodulator systems. Trace amines are believed to play a significant role in neuromodulation of synaptic transmission. The present study aimed to determine the role of trace amine-associated receptor 5 (TAAR5) in the MMN-like response in rats. First, using a bioluminescence resonance energy transfer (BRET) cAMP biosensor, we performed unbiased screening of TAAR5 ligands from a commercially available compound library (661 compounds) and identified 2-(alpha-naphthoyl)ethyltrimethylammonium iodide (alpha-NETA) as a potent (EC_{50} 150 nM) TAAR5 agonist. Then, we recorded auditory event-related potentials during an oddball paradigm in awake freely moving rats that were intraperitoneally injected with a vehicle or two doses of the putative TAAR5 agonist alpha-NETA. The MMN-like response was increased by alpha-NETA 3 mg/kg dose, but not by 1 mg/kg dose or 0.9% saline solution. These results suggest that the MMN-like response in rats may be modulated, at least in part, through TAAR5-dependent processes.

Keywords Trace amine-associated receptors · TAAR5 · 2-(Alpha-naphthoyl)ethyltrimethylammonium iodide (alpha-NETA) · Mismatch negativity (MMN) · Oddball paradigm · Awake rats

Introduction

Mismatch negativity (MMN) is an auditory event-related potential (ERP) component that reflects the automatic and pre-attentive auditory stimulus-discrimination process (Näätänen et al. 1978; Näätänen 1990; Näätänen et al. 2011). It is recorded during the oddball paradigm in which a rare change (deviant stimulus) occurs during a sequence of repeated (standard) stimuli. MMN is considered to be a result of the discordance between the deviant stimulus features and the sensory-memory representation of the standard stimulus and is associated with involuntary switching of attention to a sound change (Näätänen et al. 2005).

MMN is widely used in research on schizophrenia and is supposed to be a highly replicated biomarker (Light and Swerdlow 2015). In this category of patients, the reduced MMN amplitude is a stable indicator of auditory perception and attention abnormalities (Javitt et al. 1993; Michie 2001; Light and Braff 2005). A gradual decrease in MMN amplitude for tone frequency change reflects cognitive and functional

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impairment that depends on the duration of schizophrenia (Näätänen and Kähkönen 2009). MMN-like responses can be detected in animals; thus, MMN as a biomarker in animal models may be used for the evaluation of drug effects (Siegel et al. 2013; Harms et al. 2016).

MMN-like responses have been recorded in several animal models including monkeys (Javitt et al. 1994; Javitt et al. 1996), cats (Csépe et al. 1989, 1994), dogs (Howell et al. 2012), rabbits (Ruusuvirta et al. 1996), guinea pigs (Kraus et al. 1994), mice (Umbricht et al. 2005), and rats (Ruusuvirta et al. 1998). In rats, MMN-like responses are successfully obtained in awake, freely moving (Eriksson and Villa 2005; Roger et al. 2009; Nakamura et al. 2011; Jung et al. 2013; Harms et al. 2014) and anesthetized (Tikhonravov et al. 2010; Ahmed et al. 2011; Astikainen et al. 2011; Nakamura et al. 2011; Shiramatsu et al. 2013; Ruusuvirta et al. 2013; Mahmoudzadeh et al. 2017) animals.

MMN is a very complex process that involves several neurotransmitter systems in its generation. Pharmacological studies have revealed an important role of *N*-methyl-D-aspartate (NMDA) receptor signaling in MMN. Both human (Umbricht et al. 2000; Kreitschmann-Andermahr et al. 2001; Umbricht et al. 2002; Rosburg and Kreitschmann-Andermahr 2016) and animal model (Javitt et al. 1996; Tikhonravov et al. 2008; Harms et al. 2017) studies have shown that NMDA receptor antagonists reduce the MMN amplitude. These findings are strongly related to NMDA receptor deficits and decreased MMN amplitude in schizophrenia patients (Stephan et al. 2006; Featherstone et al. 2015).

As the role of NMDA receptors in MMN generation is mostly investigated, much less information is available about the role of other neurotransmitter systems in this process. Nicotinic receptor stimulation mostly increases MMN amplitude in humans (Engeland et al. 2002; Baldeweg et al. 2006; Dunbar et al. 2007; Dulude et al. 2010) and in animal models (Kohlhaas et al. 2015) and in some cases decreases MMN latency (Dunbar et al. 2007; Inami et al. 2005). Decreased levels of serotonin (Ahveninen et al. 2002; Kähkönen et al. 2005; Umbricht et al. 2003) or dopamine (Pekkonen et al. 1995; Kähkönen et al. 2001) cause a decrease in MMN amplitude. The role of neurotransmitters such as acetylcholine, noradrenaline, and serotonin is possibly mediated through pre- and post-synaptic neuromodulation of NMDA receptor function (Bröcher et al. 1992; Gu 2002; Baldeweg et al. 2006).

Recently, significant interest has grown concerning the investigation of the trace amine (TA)-associated receptor system. TAs are a type of biogenic amine that are present in the central nervous system of vertebrates in low concentrations (Khan and Nawaz 2016). TAs have been reported to perform neuromodulatory functions by increasing or decreasing the neuron response to neurotransmitters, predominantly serotonin and dopamine (Berry 2004). Furthermore, the pathophysiological role of TAs in several psychiatric and neurological disorders including schizophrenia, depression, attention-deficit hyperactivity, and Parkinson's disease has been estimated (Boulton 1980; Branchek and Blackburn 2003; Sherwani and Khan 2017; Pei et al. 2016). As the human TA-associated receptors (TAARs) are clustered in one of the regions associated with schizophrenia and bipolar affective disorder (Zucchi et al. 2006), the investigation of TAARs' possible roles in signaling pathways in mental disorders presents many possibilities.

TAAR1 subtype receptor is found in several brain areas and is the most studied TAAR at the moment (for review, see Miller 2011). TAAR1 plays a functional role in the regulation of brain monoaminergic systems and may therefore be implicated in the modulation of dopamine and serotonin neurotransmission (Lindemann et al. 2008; Revel et al. 2011; Miller 2011). Recently, it has been shown that TAAR1 also influences NMDA receptor-mediated glutamate transmission in the prefrontal cortex (Espinoza et al. 2015).

Much less is known about other subtypes of TAARs. Except for TAAR1, all TAAR subtypes are expressed in the olfactory epithelium, with TAAR5 having the highest expression levels (Liberles 2009; Wallrabenstein et al. 2013). However, Dinter et al. (2015) recently reported overlapping localization of mouse TAAR1 and TAAR5 in the amygdala, arcuate nucleus, and ventromedial hypothalamus. Furthermore, TAAR5 is found to be a target of 3-iodothyronamine (Dinter et al. 2015), a neuromodulator that affects adrenergic and histaminergic neurons and improves learning and memory (Zucchi et al. 2014). In this regard, better understanding of TAAR5's functional role may not only uncover the physiological relevance of TAAR5 but also provide an opportunity to evaluate the role of this receptor in pathological conditions.

In the present study, we identified 2-(alpha-naphthoyl)ethyltrimethylammonium iodide (alpha-NETA) as a potent TAAR5 agonist and tested the effect of this compound at two doses on the MMN-like response recorded in awake freely moving rats.

Methods

Screening for TAAR5 Ligands by the BRET Approach In Vitro

For identification of putative TAAR5 ligands, we performed screening of compounds from commercially available compound libraries (The SCREEN-WELL® neurotransmitter library, Enzo Life Sciences) containing 661 compounds on cAMP responses in transfected cells. For cAMP determination, we used a bioluminescence resonance energy transfer (BRET) approach with an EPAC biosensor (Barak et al. 2008). cDNA for mouse TAAR5 receptors was obtained from

Hoffmann La Roche Ltd. The gene was modified by N-terminal extension with a Rho tag and was cloned into the pcDNA3.1(+) vector. Transfections were carried out with Lipofectamine 2000 according to a standard protocol using HEK293T cells, receptor cDNA vectors, and EPAC biosensor cDNA vectors needed for evaluation of cAMP production. Then, cells were seeded into 96-well plates at 10^5 cells per well. On the following day, 70 μl of PBS was added to each well, followed by addition of 10 μl of a 50-μM coelenterazine-h solution and 10 μl of a 2-mM IBMX solution. After a 10-min incubation, either 10 μl of vehicle or 10 μl of compounds was added at a concentration of 10^{-5} M in PBS, and the plate was then placed into a Mithras LB943 instrument (Berthold Technologies, Bad Wildbad, Germany) that detects luminescent signals using a special BRET filter pair (480 nm—h-coelenterazine/RLuc and 530 nm—YFP) and MicroWin 2000 software (Berthold Technologies). The BRET signal was determined by calculating the ratio of the light emitted at 530 nm to the light emitted at 480 nm. For compounds that showed high levels of activity, EC_{50} dose-response curves were evaluated. As a control, responses to the TAAR5 agonist trimethylamine (TMA) were tested. Both agonist and antagonist activities (effects with TMA) were evaluated.

Animal Preparation

All animal studies were carried out in strict accordance with the guidelines of the Ministry of Health of Russian Federation and the principles adopted by the FELASA and RusLASA organizations of laboratory animal use. All experiments were approved by the Saint Petersburg State University Ethical Committee for Animal Research (approval number 131-03-4). All surgical procedures were performed under anesthesia, and all efforts were made to minimize suffering.

Sixteen male Wistar rats with a body weight of 220–250 g served as subjects. Rats were anesthetized with Zoletil (100 mg/kg i.p.) and xylazine (2 mg/kg i.m.); novocaine 0.5% (0.5 ml s.c.) was approved for local anesthesia.

The skin over the skull was exposed, and the epidural electrodes (gold-plated screws) were implanted through small burr holes ($d = 1.1$ mm). Active electrodes were placed above the two auditory cortices (bregma AP − 4.0, L ± 4.0) (Kohlhaas et al. 2015). The reference electrode was located at AP + 5.0, L + 0.5; the ground electrode was located at AP + 1.0, L + 1.5. The leads were fixed with dental cold-cured plastic Acrodent (JSC Ctoma, Ukraine). The operating field was treated with streptocide. Baytril (enrofloxacin, 2.5 mg/kg i.m.) was administered preoperatively as an antibiotic. The animals were allowed to recover for at least 4 days after surgery. The electrode placement was confirmed after the experiment.

Stimuli and Paradigm

Stimuli were presented in the oddball paradigm via speakers. Sounds were generated by the Psytask software (v. 2.4, Mitsar Co. Ltd., Russia). Standard and deviant sinusoidal tones were 100 ms in length (including 5 ms of rise/fall time), and the intensity was 86 dB SPL averaged across the experimental chamber. Standards were 6000 Hz frequency tones (probability 85%), and deviants were 8000 Hz frequency tones (probability 15%). In the oddball paradigm, 850 standards and 150 deviants were presented in a pseudo-random order such that before each deviant stimulus, at least one standard stimulus was presented.

Experimental Design

Each animal experiment was conducted with a 1-day break within an experimental chamber of transparent Plexiglas (length 30 cm, width 30 cm, height 25 cm) with free access to water. The chamber was located between the speakers. The awake rats were placed in the experimental chamber for 15 min before the ERP session and were free to explore during recordings.

After that, the first ERP recording of the oddball paradigm was performed; alpha-NETA (1 mg/kg in low-dose condition or 3 mg/kg in high-dose condition; volume of injection 1 ml/kg, i.p.) was administered intraperitoneally 15 min prior to the start of the second ERP recording session. The drug was prepared in 0.9% saline solution before the injection. An injection of 0.9% NaCl vehicle was used in the control condition. The order of conditions was counterbalanced across animals. Electrocorticogram (ECoG) was recorded continuously during the experiment; a Mitsar-EEG-05/70-201 amplifier and WinEEG software were used (v. 2.4, Mitsar Co. Ltd., Russia).

Data Analysis

The ECoG was filtered (bandpass 0.32–50 Hz), and artifacts were removed by visual inspection during offline analysis. High frequency (100–200 Hz) and high amplitude waves (amplitude exceeds ± 500 μV on any of the channels) occurred primarily during grooming (Roger et al., 2009) and were rejected. For each animal, separately average evoked potentials were calculated for standard and deviant stimuli before and after drug administration. Baseline data were collected from − 100 to 0 ms before stimulus onset. Mismatch responses were calculated as the deviant minus standard event-related potential.

The ERP was characterized by an early negative peak at 20 ms (N1), a positive peak at 32 ms (P1), and a negative peak at 44 ms (N2), followed by the broad late positive component (P2) with two discernible peaks at approximately 64 and 84 ms. Mean latency and amplitude measures (baseline to

peak) were extracted over latency windows corresponding to the ERP peaks: 8-ms windows from 16 to 24, 26 to 32, and 40 to 48 ms for N1, P1, and N2, respectively, and a 60-ms window from 60 to 120 ms for P2. Mean latencies and amplitudes were obtained for standard, deviant, and difference (mismatch) responses.

Statistical Analysis

All data were normally distributed according to the Kolmogorov-Smirnov test. Mean latencies and amplitudes of the ERP components in all three conditions were analyzed using repeated measures analysis of variance (ANOVA) with the factors *injection* (before and after), *stimulus type* (deviant and standard), and *electrode location* (left and right). The Greenhouse-Geisser correction was used where sphericity assumptions were violated. The paired samples *t* test was performed in case a significant main effect or interaction was found.

Results

Identification of a Putative TAAR5 Agonist

To date, only trimethylamine and 3-iodothyronamine have been identified as TAAR5 ligands (Wallrabenstein et al. 2013; Dinter et al. 2015). Because TAAR5 is a G_{olf}-coupled receptor and stimulates production of cAMP, we performed drug screening for TAAR5 activation by analyzing cAMP accumulation. In all, 661 compounds including neurotransmitters, cholinergic, GABAergic, histaminergic, serotonergic, and metabotropic glutamatergic ligands have been tested in our BRET cAMP assay (Online resource 1). Among them, only 2-(alpha-naphthoyl)ethyltrimethylammonium iodide (alpha-NETA) gave a positive signal as an agonist of TAAR5. The dose-response experiments (Fig. 1) have shown that the effective concentration of alpha-NETA was approximately 150 nM. By comparison, the effective TMA concentration was found to be 230 nM. The potency of alpha-NETA in comparison to TMA was 115%. No effects of these compounds at the same concentrations tested were found in cells not expressing TAAR5 or cells expressing mouse or human TAAR1 (data not shown). We emphasize that while this compound incorporates tertiary amine groups such as choline, there was no response from other choline-like drugs tested. Thus, we identified a new prospective chemotype of TAAR5 receptor agonist. Since it was shown previously that alpha-NETA has reasonable pharmacokinetic properties and safety profile and likely passes the blood-brain barrier as evidenced by the effectiveness in animal models of autoimmune CNS inflammation following systemic administration (Graham et al. 2014), we evaluated the effect of this compound in our

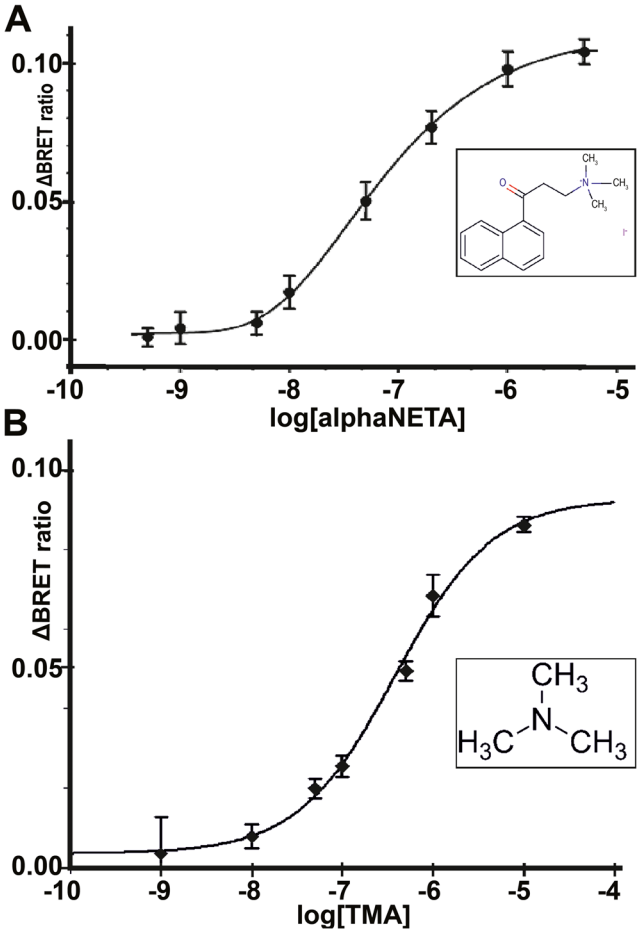


Fig. 1 Dose-response curve for alpha-NETA (**a**) and TMA (**b**) on cAMP BRET responses and their chemical structures (inserts). At least six independent experiments were performed for each compound

in vivo experimental paradigm. Notably, since in this study, alpha-NETA was administered chronically at a dose of 10 mg/kg, s.c., we elected to use lower doses of 1 and 3 mg/kg in our study.

Deviance Detection

Deviance detection in all paradigms was confirmed by a significant difference between deviant and standard responses in the late P2 potential. There was a significant main effect of *stimulus type* for control ($F(1,14) = 24.707$; $p < 0.001$), low-dose ($F(1,14) = 16.043$; $p = 0.002$), and high-dose ($F(1,14) = 28.681$; $p < 0.001$) conditions before and after injection at latency values from 60 to 120. No effects were found for the early N1, P1, or N2 potentials. No significant differences in the latencies of any components were observed between the responses to the standard and the deviant tones in any of the experimental conditions. Standard and deviant potentials before injection in the control condition are presented in Fig. 2.

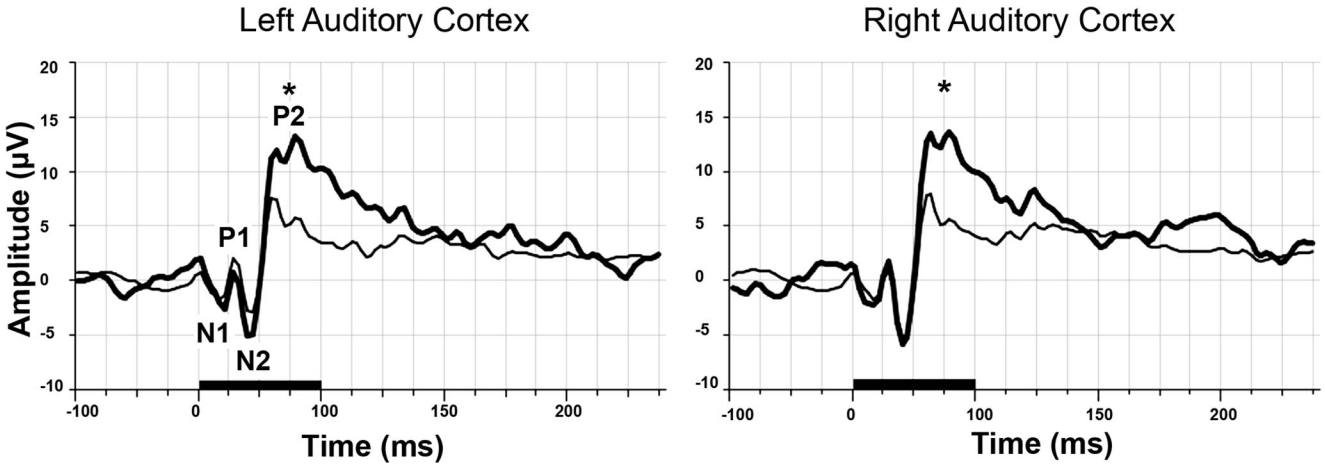


Fig. 2 Grand average ($N = 16$) standard (thin line) and deviant (thick line) responses for electrodes implanted above the left and right auditory cortex before injections of 0.9% NaCl. Black bar on the *x*-axis shows the stimulus duration. Asterisks denote significant differences ($p < 0.05$) between standard and deviant responses in the 60–120-ms time range

Effect of Drug Injection on N1, P1, and N2 Components

In the control and alpha-NETA low-dose conditions, no significant effects or interactions on N1 amplitude were revealed. In the high-dose condition, significant *injection* × *electrode location* ($F(1,14) = 9.379$; $p = 0.010$) and *injection* × *stimulus type* × *electrode location* ($F(1,14) = 8.372$; $p = 0.013$) interactions for the N2 amplitude were found. Paired samples *t* tests indicated an increased amplitude of the deviant potential above the left auditory cortex compared to the right ($t = 2256$; $p = 0.043$) before the injection. After the injection, that potential was reduced ($t = -2349$; $p = 0.037$). Difference (MMN) amplitudes (Fig. 3) were also significantly larger on the left side of the auditory cortex ($t = 2653$; $p = 0.021$) and also decreased after injection ($t = -2706$; $p = 0.019$).

For the P1 component, a main effect of *electrode location* ($F(1,14) = 186.979$; $p = 0.010$) was found in the low-dose alpha-NETA condition. Before the injection, the amplitude of the response was greater on the left side for standard ($t = 2.762$; $p = 0.017$) and deviant ($t = 3.159$; $p = 0.008$) stimuli. No difference was found for the MMN-like response amplitude.

For the N2 component, a significant main effect of *injection* ($F(1,14) = 4909$; $p = 0.042$) and *injection* × *stimulus type* interaction ($F(1,14) = 5159$; $p = 0.037$) were found in the control condition: the amplitude of the deviant potential above the left auditory cortex decreased after the 0.9% NaCl injection ($t = 2.733$; $p = 0.015$). There was also a significant decrease in the 40–48-ms range after the NaCl injection above the left auditory cortex ($t = 2293$; $p = 0.041$) for the difference amplitudes. In the low-dose condition, a significant *electrode location* effect ($F(1,14) = 8641$; $p = 0.009$) was found: N2 amplitudes above the left auditory cortex were larger in response to standard stimuli before ($t = 2.811$; $p = 0.012$) and after ($t = 3158$ $p = 0.006$) injection and in response to deviant stimuli after injection ($t = 2.264$; $p = 0.037$). No significant effects or interactions were found in the high-dose condition for the N2 component or difference wave.

Effect of Drug Injection on the P2 Component

In control and alpha-NETA low-dose conditions, no significant *injection* main effect or interactions on P2 amplitude were revealed.

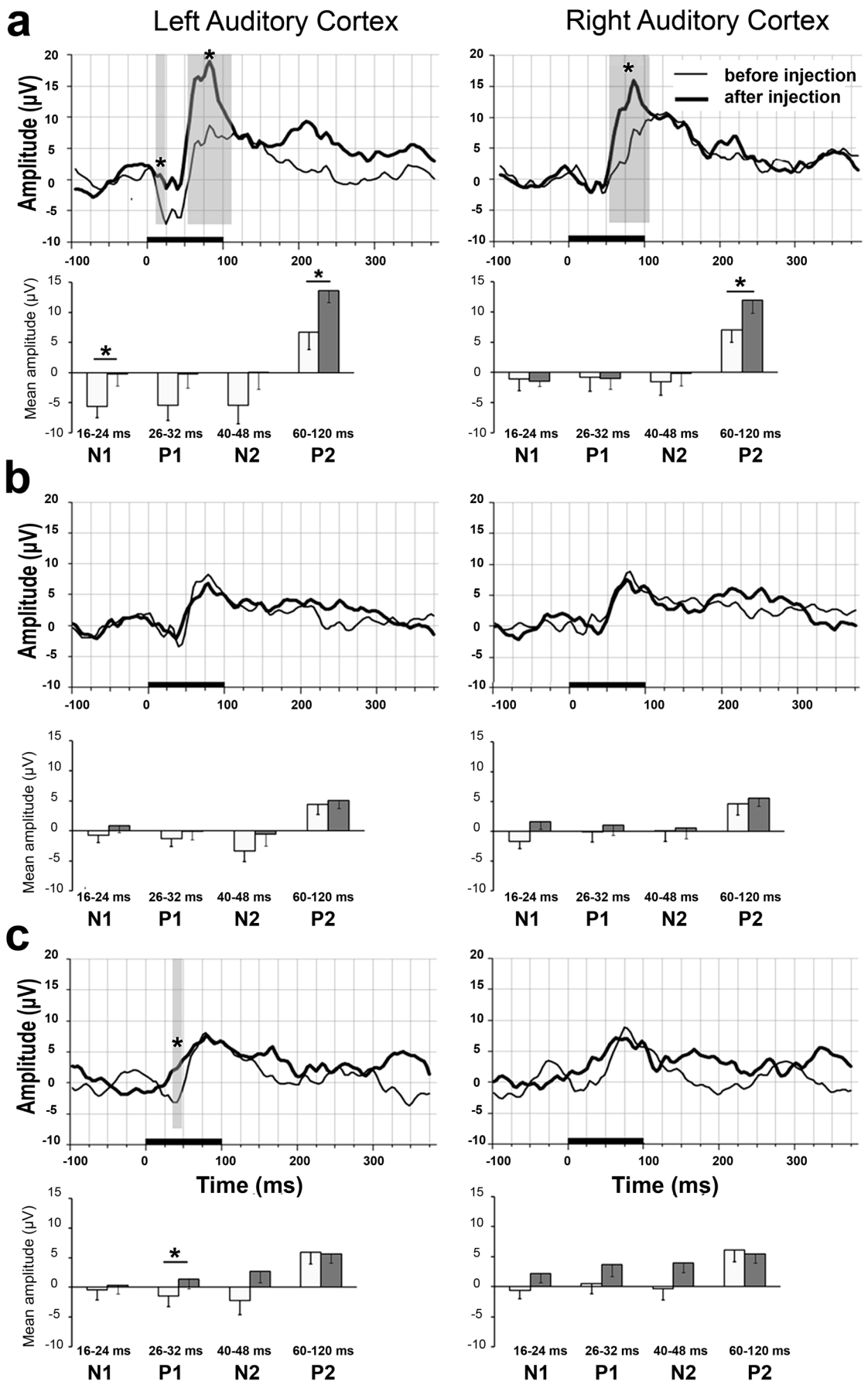
In the high-dose condition, a significant *injection* effect ($F(1,14) = 5.409$; $p = 0.038$) and an *injection* × *stimulus type* interaction ($F(1,14) = 6.110$; $p = 0.029$) at 60–120 ms were found. Paired samples *t* tests indicated an increased amplitude of the deviant potential (left: $t = 2.594$; $p = 0.023$; right: $t = 2.225$; $p = 0.046$) and no difference in the standard potential.

Difference amplitudes (Fig. 3) also significantly increased after high-dose injection of alpha-NETA ($F(1,14) = 6.962$; $p = 0.022$) in the 60–120-ms range. They were significantly larger after the alpha-NETA high-dose administration than after the alpha-NETA low-dose (left: $t = 4731$; $p < 0.001$; right: $t = 3.693$; $p = 0.003$) and 0.9% NaCl (left: $t = 2.684$; $p < 0.020$; right: $t = 2.233$; $p = 0.045$) administrations.

No effects on the latency of any described components after injections in the control, low-dose, or high-dose alpha-NETA conditions were found.

Discussion

This study aimed to evaluate the effect of a newly identified putative TAAR5 agonist, alpha-NETA, on MMN-like responses recorded during an oddball paradigm in freely moving rats.


a
Left Auditory Cortex
Right Auditory Cortex
before injection
after injection
Amplitude (μV)
Mean amplitude (μV)
16-24 ms
26-32 ms
40-48 ms
60-120 ms
N1
P1
N2
P2
b
c
Time (ms)

◀ **Fig. 3** Grand average ($N = 16$) MMN-like activity displayed as difference waveforms for electrodes implanted above the left and right auditory cortex before (thin line) and after (thick line) injections of high-dose alpha-NETA (3 mg/kg) (**a**), low-dose alpha-NETA (1 mg/kg) (**b**), or 0.9% NaCl (**c**). The black bar on the *x*-axis shows the stimulus duration. Asterisks denote significant differences ($p < 0.05$) between ERPs before and after injection in the gray-shaded latency range. Mean amplitudes (± standard error, SE) of difference waveforms in corresponding latency windows for N1, P1, N2, and P2 before (white columns) and after (gray columns) injections are shown under the ERPs. Asterisks denote significant difference ($p < 0.05$)

Evidence of MMN-like response latency and polarity are quite variable among studies in rats. These characteristics strongly depend on the reference electrode position and anesthetic agents (Harms et al. 2016). The majority of studies indicate early and late components of the acoustic ERP in rats with a deviance detection-like shift independent from adaptation and peaking between 50 and 100 ms with positive (Ahmed et al. 2011; Nakamura et al. 2011; Astikainen et al. 2011; Jung et al. 2013; Ruusuvirta et al. 2015) or negative (Nakamura et al. 2011; Harms et al. 2014; Shiramatsu et al. 2013; Sivarao et al. 2014) polarity.

We revealed four components of auditory ERPs in all conditions. The peak latencies of N1 (mean 20 ms), P1 (mean 32 ms), N2 (mean 44 ms), and P2 (mean 74 ms) components in response to standard and deviant stimuli were highly similar to components reported by Jung et al. (2013) in polarity and peak latencies. In our study, the difference (MMN-like) response had early negative and late positive shifts; the significant difference between the standard and deviant stimuli peaked at approximately 80 ms, indicating that deviance detection was reflected in the P2 component. We revealed asymmetry in the amplitude of the early components. Increased N1, P2, and N2 potentials over the left auditory cortex occurred as a result of the reference electrode position: it was located at the left side of the sagittal line (AP + 5.0, L + 0.5) in order not to damage the venous sinus.

The present study found that MMN-like ERPs in rats were sensitive to alpha-NETA. The early N1 component in response to the deviant stimuli and the difference wave in the corresponding time window were decreased after a high-dose alpha-NETA injection. However, in the control condition, a similar effect was seen from an injection of 0.9% NaCl on the early N2 component and difference wave at 40–48 ms. Amplitudes of auditory ERPs could decrease from the influence of stress (Karl et al. 2006) or fatigue (Evstigneeva et al. 2010). Therefore, this finding may be attributed to the effect of post-injection stress rather than to the effect of the drug.

In contrast to the early components, the late components of the difference wave did not differ before or after an injection of NaCl or a low dose of alpha-NETA. In the high-dose alpha-NETA condition, a significant difference was found between the difference wave before and after the injection within the 60–120-ms time window. This finding suggested that the high dose (3 mg/kg, i.p.) of alpha-NETA had a stronger influence on the MMN-like activity than the low dose (1 mg/kg, i.p.) of the drug.

It should be noted that alpha-NETA had been known for a long time to be an inhibitor of acetylcholine-synthesizing enzyme choline acetyltransferase (ChAT) at relatively high concentrations with an IC_{50} of 9 μM (Kumar et al. 2016, but see also Martin et al. 2009) and thus may potentially cause a decrease in acetylcholine levels (Sastry et al. 1988). Since the stimulation of acetylcholine nicotinic receptors improved stimulus encoding and increased the MMN response in humans (Baldeweg et al. 2006; Dunbar et al. 2007; Martin et al. 2009; Fisher et al. 2012) and rats (Kohlhaas et al. 2015), it is unlikely that the increased MMN-like response reported in our study resulted from ChAT inhibition. Conversely, alpha-NETA injection leads to a reaction that is similar to nicotine injection, which suggests similarity between nicotinic receptors (Bröcher et al. 1992; Gu 2002) and TAAR1 (Espinoza et al. 2015). TAAR5 may have a neuromodulatory function (possibly on NMDA receptors). It should also be noted that alpha-NETA affected MMN-like electrophysiological activity 15 min after administration, which was consistent with the non-enzymatic effect of this compound because enzyme inhibition generally requires a longer period of time to decrease enzymatic activity in comparison to action on other targets (e.g., G-protein-coupled receptor (GPCR) activation). Among other known targets (out of 102 evaluated), alpha-NETA was the most potent at inhibiting aldehyde dehydrogenase 1 family, member A1 (ALDH1A1) with an IC_{50} of 40 nM, followed by GPCR chemokine-like receptor-1 (CMKLR1) with an IC_{50} of 380 nM (Graham et al. 2014), but the role of these targets on the effects observed is unclear. Nevertheless, alpha-NETA is the most active TAAR5 agonist known to date with an EC_{50} of 150 nM and exerts the highest activity on TAAR5 among other GPCR targets, thus providing an opportunity to further investigate the functional role of TAAR5 in higher cognitive processes or other physiological functions. Finally, since TAAR5 is found in mouse leukocytes (Nelson et al. 2007), it would be important to evaluate the role of TAAR5 activation in recently demonstrated alpha-NETA's effectiveness in animal models of autoimmune CNS inflammation that likely involves an altered leukocyte trafficking (Graham et al. 2014).

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Compliance with Ethical Standards

All animal studies were carried out in strict accordance with the guidelines of the Ministry of Health of Russian Federation and the principles adopted by the FELASA and RusLASA organizations of laboratory animal use. All experiments were approved by the Saint Petersburg State University Ethical Committee for Animal Research (approval number 131-03-4). All surgical procedures were performed under anesthesia, and all efforts were made to minimize suffering.

Conflict of Interest The authors declare that they have no conflict of interest.

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